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mTORC1: Turning Off Is Just as Important as Turning On

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mTORC1 is activated primarily on the lysosome. Menon et al. and Demetriades et al. show that mTORC1 deactivation on the lysosome is determined by recruitment of its negative regulator, the tumor suppressor complex TSC1-TSC2. These reports highlight the importance of subcellular localization in the regulation of mTORC1.

The mammalian target of rapamycin complex 1 (mTORC1) is a master regulator of cell growth that integrates multiple inputs such as growth factors and nutrients (positive inputs), and hypoxia, endoplasmic reticulum (ER) stress, and low energy levels (negative inputs). Almost all of these inputs act on the TSC complex (comprising tuberous sclerosis complex 1, TSC2, and TBC1D7), an upstream inhibitor of mTORC1. The TSC complex is a GTPase-activating protein (GAP) for the Rheb GTPase, which is the immediate upstream activator of mTORC1. The activation/inactivation of the TSC complex in response to relevant signals inversely correlates with Rheb activity and, by extension, with mTORC1 activity. An exception to the TSC-Rheb-mTORC1 regulatory axis is the sensing of intracellular amino acid levels. Amino acid sufficiency is sensed at the lysosome, which is the focal point for mTORC1 activation in the cell. Key players in amino acid sensing are the Rag GTPases (Sancak et al., 2008; Kim et al., 2008). Heterodimers of RagA or B with RagC or D acquire their active guanyl nucleotide-loaded configuration (RagA/B^{GTP}-RagC/D^{GDP}) in the presence of amino acids and recruit mTORC1 to the lysosome. At the lysosome, mTORC1 encounters Rheb, which appears to be constitutively present on the lysosomal surface (Figure 1) (Bar-Peled and Sabatini, 2012; Betz and Hall, 2013). Importantly, mTORC1 is activated only if Rheb is activated by upstream growth factor signaling, indicating that, although both amino acids and growth factors are necessary, neither alone is sufficient to activate mTORC1. Two papers in this issue of *Cell* from Menon et al. (2014) and Demetriades et al. (2014) provide additional insight on the regulation of mTORC1, in particular on how mTORC1 signaling is shut off in the absence of amino acids. The authors suggest that translocation of the TSC complex to the lysosome is the major determinant of mTORC1 inactivation.

Menon et al. (2014) approach mTORC1 regulation from the angle of growth factor signaling. It was known that growth-factor-stimulated Akt activates mTORC1 by phosphorylating and thereby somehow inactivating TSC2. Menon et al. (2014) now show that Akt phosphorylation causes the dissociation of TSC2 from the lysosome, such that it is no longer able to inactivate Rheb. Akt phosphorylation of TSC2 does not result in a significant reduction in intrinsic TSC2 GAP activity or in TSC2 protein stability or TSC complex stability as previously thought. Rather, it is the to and fro shuttling of the mTORC1 repressing TSC complex from the lysosome, shown to be under the control of growth factor signaling, that is responsible for growth factor regulation of Rheb and mTORC1 activity.

Demetriades et al. (2014) concentrate on amino acid activation of mTORC1 and make the surprising discovery that the Rags are required for mTORC1 deactivation following amino acid removal. As shown previously, amino acid sufficiency leads the Rags to acquire an activated guanyl-loaded configuration and thereby to recruit mTORC1 to the lysosome. In contrast, absence of amino acids leads the Rags to adopt an inactive configuration (RagA/B^{GDP}-RagC/D^{GTP}) that is unable to recruit mTORC1. Demetriades et al. (2014) now show that, in this "inactive" form, the Rag heterodimer binds and recruits the TSC complex to the lysosome to inhibit Rheb and mTORC1 signaling. Thus, depending on amino acid availability, the Rags have either a positive or a negative role in regulating mTORC1.

Importantly, both studies suggest that mTORC1 deactivation is an acute, active process and not merely a dampening of mTORC1 signaling due to dissipation of its upstream stimuli. This requirement for mTORC1 signaling to be abruptly turned off when no longer needed demonstrates that mTORC1 activity is tightly controlled and suggests a corresponding level of control for mTORC1 downstream effectors such as the S6K, 4EBP, and ULK1 proteins. Dephosphorylation of these proteins and whether their respective phosphatases are positively stimulated under conditions where mTORC1 becomes inactivated may be a rewarding field for future studies.

There are some discrepancies between the two reports. Menon et al. (2014) do not observe any significant differences in lysosomal localization of TSC2 in response to amino acids. They also find that, in the absence of amino acids, when TSC should be recruited to the lysosome by "inactive" Rag, insulin stimulation causes translocation of TSC2 away from the lysosome. Whether this is due to a difference in cell types or to technical differences remains to be seen. A

possible explanation underlying cell-type-specific differences may be that the amount of Rag proteins is limiting in some cell types. As previously mentioned, the two different forms of Rag heterodimers act antagonistically in the regulation of mTORC1. In cells in which the number of Rag complexes is limiting, formation of RagA/B^{GTP}-RagC/D^{GDP} heterodimers comes at the expense of RagA/B^{GDP}-RagC/D^{GTP} heterodimers in amino-acid-replete conditions and vice versa under amino acid deprivation. In this situation, one form of the heterodimer can rapidly dominate over the other, making the system finely tuned to respond to amino acid levels. Conversely, in cells expressing an excess of Rag proteins, both heterodimeric forms may coexist at an equilibrium that would require the outside intervention of growth factor signaling to tip the balance in otherwise sluggish regulation. In such a scenario, differences in the duration of amino acid starvation or in basal amino acid levels could conceivably give rise to seemingly discordant observations. Whatever the explanation, the role of Akt-mediated TSC2 phosphorylation in Rag-mediated TSC recruitment needs to be clarified.

A recent report (Zhang et al., 2013; Benjamin and Hall, 2013) described the TSC1-TSC2 complex and Rheb on the peroxisome, where they regulate mTORC1 activity in response to peroxisomal ROS and growth factors. In this case, TSC2 phosphorylation by insulin stimulated Akt results in TSC2 translocation from the peroxisome to the cytosol, leading to mTORC1 activation in a manner reminiscent of the reports presented here. Thus, regulation of peroxisomal TSC may provide a precedent that functionally links TSC2 phosphorylation and its localization. However, Menon et al. (2014) were not able to detect TSC2 at the peroxisome. Clearly, the TSC complex will continue to be the focus of many more fascinating studies.

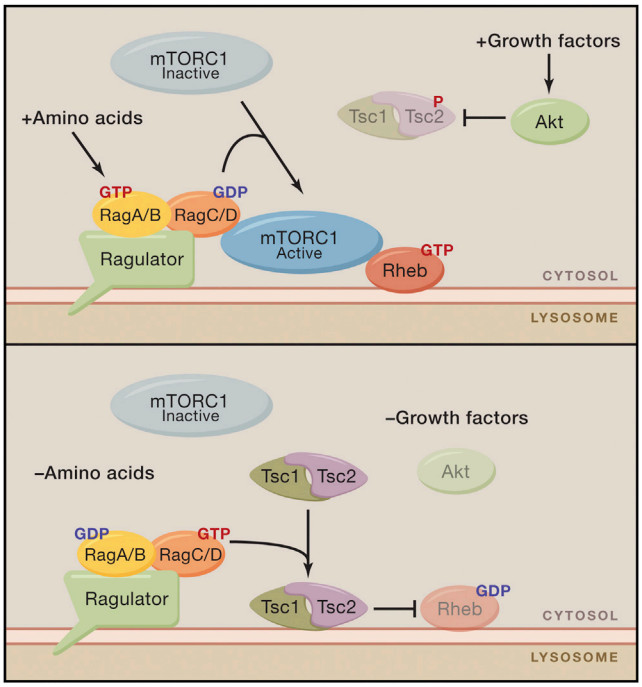

Figure 1. Amino Acid and Growth Factor Signaling Converge on the Lysosome to Control mTORC1

Amino acids induce the Rag heterodimer to acquire the RagA/B^{GTP}-RagC/D^{GDP} configuration. This form of the Rag heterodimer recruits mTORC1 to the lysosomal surface. Once on the lysosomal surface, mTORC1 is activated by growth-factor-stimulated Rheb. Growth factors stimulate Rheb via Akt-mediated phosphorylation and inhibition of TSC2. In the absence of amino acids, the Rags adopt the RagA/B^{GDP}-RagC/D^{GTP} configuration that recruits the TSC complex to the lysosome. The TSC complex is a GTPase-activating complex that inactivates Rheb and, in turn, mTORC1 signaling.

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